Title: Hydrolysable hydrogels for controlled release.

The present invention relates to hydrogels which have a good controlled release behaviour, and to processes to prepare such hydrogels.

The fast developments in the field of molecular

5   biology and biotechnology have made it possible to produce a large number of pharmaceutically interesting products in large quantities. For instance, pharmaceutically active peptides and proteins can suitably be used as drugs in the treatment of life-threatening diseases, e.g. cancer, and

10   of several types of viral, bacterial and parasital diseases; in the treatment of e.g. diabetes; in vaccines, e.g. for prophylactic aims, and for anticonception purposes. Especially the specialized biological activities of these types of drugs provide tremendous advantages over

15   other types of pharmaceutics.

To illustrate the fast developments, it has been reported (see e.g. Soeterboek and Verheggen, Pharm. Weekblad 130 (1995) 670-675) that in the United States of America, about 275 biotechnological products are in phase

20   IV studies, while more than 500 products are under investigation.

Examples of (recombinant) proteins, which are considered very interesting from a pharmacological point of view, are cytokines, such as interleukines,

25   interferons, tumor necrosis factor (TNF), insulin, proteins for use in vaccines, and growth hormones.

Due to their nature, proteins and proteinaceous products, including peptides, which group of products will be referred to as protein drugs herein-below, cannot be

30   administered orally. These products tend to degrade rapidly in the gastro-intestinal tract, in particular because of the acidic environment and the presence of proteolytic enzymes therein.

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Moreover, to a high extent protein drugs are not able
to pass endothelial and epithelial barriers, due to their
size and, generally, polar character.

For these reasons, protein drugs have to be brought
5   in the system parenterally, i.e. by injection. The
pharmacokinetical profile of these products is, however,
such that injection of the product per se requires a
frequent administration. For, it is a known fact that
proteinaceous material is eliminated from the blood
10   circulation within minutes.

In other words, since protein drugs are chemically
and/or physically unstable and generally have a short
half-time in the human or animal body, multiple daily
injections or continuous infusions are required for the
15   protein drug to have a desired therapeutic effect. It will
be evident that this is inconvenient for patients
requiring these protein drugs. Furthermore, this type of
application often requires hospitabilization and has
logistic drawbacks.

20   In addition, it appears that at least for certain
classes of pharmaceutical proteins, such as cytokines
which are presently used in e.g. cancer treatments, the
therapeutic efficacy is strongly dependent on effective
delivery, e.g. intra- or peritumoral. In such cases, the
25   protein drugs should be directed to the sites where their
activity is needed during a prolonged period of time.

Hence, there is a need for delivery systems which
have the capacity for controlled release. In the art,
delivery systems consisting of polymeric networks in which
30   the proteins are loaded and from which they are gradually
released have been proposed.

More in detail, at present, two major types of
polymeric delivery systems can be distinguished:
biodegradable polymers and non-biodegradable hydrogels.
35   Biodegradable polymers, e.g. polylactic acid (PLA)
and copolymers of PLA with glycolic acid (PLGA), are
frequently used as delivery systems for proteins.

Proteins can be incorporated in pharmaceutical delivery systems, e.g. microspheres, by a variety of processes. *In vitro* and *in vivo*, usually a biphasic release profile is observed: an initial burst followed by

5 a more gradual release. The burst is caused by proteinaceous material present at or near the surface of the microspheres and by proteinaceous material present in pores. The gradual release is ascribed to a combination of diffusion of the proteinaceous material through the matrix

10 and degradation of the matrix. Especially for larger proteins diffusion in these matrices is negligible, so that the release depends on the degradation of the polymer. The degradation can be influenced by the (co)polymer composition. A well-known strategy to increase

15 the degradation rate of PLA is co-polymerization with glycolic acid.

Although delivery systems based on biodegradable polymers are interesting, it is very difficult to control the release of the incorporated protein. This hampers the

20 applicability of these systems, especially for proteins with a narrow therapeutic window, such as cytokines and hormones. Furthermore, organic solvents have to be used for the encapsulation of the protein in these polymeric systems. Exposure of proteins to organic solvents

25 generally leads to denaturation, which will affect the biological activity of the protein. Furthermore, the very stringent requirements of registration authorities with respect to possible traces of harmful substances may prohibit the use of such formulations of therapeutic drugs

30 in human patients.

Also hydrogels are frequently used as delivery systems for proteins and peptides. Hydrogels can be obtained by crosslinking a water-soluble polymer yielding a three-dimensional network which can contain large

35 amounts of water. Proteins can be loaded into the gel by adding the protein to the polymer before the crosslinking reaction is carried out or by soaking a preformed hydrogel

in a protein solution. So, no (aggressive) organic
solvents have to be used to load the hydrogels with
protein molecules.

5   In contrast with the biodegradable polymers, the
release of proteins from hydrogels can be easily
controlled and manipulated by varying the hydrogel
characteristics, such as the water content and the
crosslink density of the gel. However, a major
disadvantage of the currently used hydrogel delivery
10   systems is that they are not biodegradable. This
necessitates surgical removal of the gel from the patient
after the release of the protein in order to prevent
complications of inclusion of the empty hydrogel material
(wound tissue is frequently formed).

15   Biodegradable hydrogels have been used in the
preparation of delivery systems for protein drugs. One of
these systems comprises crosslinked dextrans obtained by
coupling glycidyl methacrylate (GMA) to dextran, followed
by radical polymerization of an aqueous solution of GMA-
20   derivatized dextran (dex-GMA). In this respect, reference
is made to Van Dijk-Wolthuis et al. in Macromolecules 28,
(1995), 6317-6322 and to De Smedt et al. in Macromolecules
28, (1995) 5082-5088.

Proteins can be encapsulated in the hydrogels by
25   adding proteins to a solution of GMA-derivatized dextran
prior to the crosslinking reaction. It appeared that the
release of the proteins out of these hydrogels depends on
and can be controlled by the degree of crosslinking and
the water content of the gel (Hennink et al., J. Contr.
30   Rel. 39, (1996), 47-57).

Although the described crosslinked dextran hydrogels
were expected to be biodegradable, these hydrogels are
rather stable under physiological conditions. This can is
further elaborated in Example 5. It is shown among other
35   that the dissolution time of dextran hydrogels obtained by
polymerization of dextran derivatized with glycidyl
methacrylate (DS = 4) had a dissolution time of about 100

days. Dextran hydrogels, wherein the dextrans have a
higher degree of substitution, did not show any signs of
degradation during 70 days, even at extreme conditions.

The object of the present invention is to provide a
5   slow or controlled release delivery system which does not
possess the above-mentioned disadvantages, and especially
does not require the use of organic solvents, does not
show the undesired and uncontrollable burst effects, and
do not possess a poorly controllable release behaviour.
10   The present invention aims to combine the advantages of
both types of known delivery systems, viz. a system,
(bio)degradable under physiological conditions - either
chemically and/or enzymatically -, with controlled protein
drug release.
15   The present invention provides safe and easily
controllable delivery systems, based on particular
biodegradable hydrogels, which increase the applicability
of protein drugs for the treatment of various diseases.
The risks associated with these drugs, such as bursts in
20   the release profile, and the inconvenience for the patient
are reduced, while the therapeutic efficacy of drug
treatments using the hydrogels of the present invention is
increased.

More in detail, the present invention relates to a
25   biodegradable hydrogel comprising bonds which are
hydrolysable under physiological conditions. The hydrogels
of the present invention contain hydrolytically labile
spacers, which spacers are broken in human or animal
bodies. As roughly indicated herein-above, a hydrogel is
30   defined as a water-swollen, three-dimensional network of
crosslinked hydrophilic macromolecules. More in detail,
the hydrogels of the invention consist of two
interpenetrating networks interconnected to one another
through hydrolysable spacers. Hydrogels generally contain
35   from 20 to more than 99 wt.% water.

Further, the invention relates to a method for the
preparation of a hydrogel, wherein macromolecules, e.g.

polymers, which contain bonds which are hydrolysable under
physiological conditions, are crosslinked in an aqueous
solution.

5   In accordance with the present invention, all types
of biodegradable hydrogels can be used, provided that
hydrolytically labile spacers can be introduced in these
structures. When brought into the system of an animal or
human body, the hydrogel structure is more or less
gradually broken down. The degradation products do not

10   need to be removed after therapy; they can simply be
metabolized and/or excreted by the body.

Preferably, the hydrogels are based on water soluble
polymers which contain at least a number of side groups
having the capability to form linkers to other polymers,

15   e.g. dextran or derivatized dextrans, while starches and
starch derivatives, cellulose derivatives such as
hydroxyethyl and hydroxypropyl cellulose,
polyvinylpyrrolidone, proteins, polyamino acids,
polyvinylalcohol, polyacrylates, polymethacrylates,

20   polyethylene glycols that contain a number of monomer
units capable of forming side chains, and so on, can also
be used, as well as copolymers thereof.

The hydrogels of the invention are suitably based on
a polymer crosslinked with methacrylate units. Other

25   crosslinking units are acrylate units, vinyl ethers and
vinyl esters, as well as other units known for this
purpose by the person skilled in the art.

Generally, the water-swellable polymers used in the
present invention are made hydrolysable by introducing at

30   least one hydrolytically labile unit in the spacers
between the main chains of water soluble polymers and the
second polymer chain formed by the crosslinkable units as
described in the preceding paragraph. It is also possible
to use polymer chains comprising hydrolytically labile

35   monomer units in the main chain. However, not within the
scope of the present invention are hydrogels which only
contain polymer chains that are interconnected head-to-

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tail only by hydrolyzable spacer groups. Contrary to
hydrogels according to the invention, in hydrogels based
on swellable polymers which are only substituted with
labile spacers head-to-tail, the degree of crosslinking is
5   directly correlated to the water content of the gel. The
polymer system the hydrogel of the invention, makes it
possible to control the release of compund by adjusting
the water content and/or the degree of crosslinking
independently.

10   In a preferred embodiment, the hydrogels of the
present invention comprise as hydrolytically labile units
hydrolysable lactate and/or carbonate ester bonds. These
bonds can be brought into the hydrogel by introducing e.g.
(poly)glycolic acid and/or (poly)lactic acid residues
15   between the main chain of the polymer and the
crosslinkable groups of said polymer. With the term
(poly)glycolic acid, glycolic acid as well as di- and
oligomers thereof are meant. With the term (poly)lactic
acid, lactic acid as well as di- and oligomers thereof are
20   meant. Other possibilities for hydrolytically labile units
are based on units introducing carboxylic esters,
urethanes, anhydrides, (hemi)acetals, amides and peptide
bonds.

In the most preferred embodiment of the present
25   invention, (poly)glycolic acid and/or (poly)lactic acid
spacers are introduced between polymerizable methacrylate
groups and dextran. When a hydrogel formed of this
material is introduced in a physiological environment, the
hydrogel becomes biodegradable resulting in dextran,
30   polyhydroxyethylmethacrylate (PHEMA), lactic acid and/or
glycolic acid as degradation products. These degradation
products are all biocompatible. It is noted that lactic
acid and glycolic acid are endogenous compounds. Dextran
is a non-toxic polymer, which is used for many years as
35   plasma expander and is cleared by the kidneys depending on
its molecular weight. PHEMA is a well-known biocompatible

polymer, which is probably cleared via the kidneys, as
well.

   Hydrogels of the present invention can suitably be
prepared by first synthesizing spacers which contain at
5 least one crosslinkable group and at least one
hydrolytically labile group; coupling such spacers to a
water-soluble polymer, and crosslinking the polymers
obtained, preferably in the presence of the compound to be
released.

10   Preferred spacers within the present invention
comprise a hydroxyethyl methacrylate (HEMA) group, coupled
to one or more lactide and/or glycolide units, as
exemplified in steps a and b of scheme 1. More in detail,
HEMA-terminated polylactic acid pre-polymers can be
15 synthesized by solution polymerization of lactide in
toluene using HEMA as initiator and aluminium alkoxide as
catalyst or by a bulk polymerization of lactide using HEMA
as initiator and stannous octoate as catalyst. Dependent
on the ratio HEMA/lactic acid pre-polymers which differ in
20 molecular weight of the lactic acid block can be
synthesized. HEMA terminated copolymers of glycolic acid
and of glycolic acid-co-lactic acid can be synthesized in
analogy. The pre-polymers can be characterized by known
techniques, e.g. NMR and IR spectroscopy, differential
25 scanning calorimetry (DSC), and gel permeation
chromatography (GPC). In order to couple the HEMA
terminated polylactic and/or glycolic acid pre-polymers to
dextran, the terminal hydroxyl group has to be activated.
Preferably, the binding of HEMA to dextran is effected by
30 carbonyl-di-imidazole (CDI) as coupling agent. However,
also other activation methods can be used. For example,
reaction of the hydroxyl function of the HEMA lactic acid
pre-polymer with succinic anhydride, followed by
activation of the formed carboxylic group using
35 established methods (e.g. dicyclohexylcarbodiimide (DCC)
activation). The latter method yields dextran derivatives
in which only hydrolytically instable ester bonds are

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present, which provide different degradation
characteristics compared to the dextran derivatives
synthesized with the CDI-method, in which both ester bonds
and carbonate bonds are present.

5   The activated HEMA terminated polylactic and/or
glycolic acid pre-polymers are subsequently coupled to
dextran in a suitable aprotic solvent (such as DMSO), in
the presence of a catalyst, e.g., N,N-dimethylamino-
pyridine (DMAP). The degree of substitution (i.e. number

10   of moles of methacrylate groups containing prepolymer per
100 moles glucose units of dextran) can be tailored by the
ratio of HEMA containing prepolymer versus dextran in the
reaction mixture.

        From these substituted polymers, hydrogels are

15   prepared, e.g., by a radical polymerization of an aqueous
solution of HEMA-pre-polymer functionalized dextran using
a known initiator system of a tertiary amine and a
persulphate (see e.g. the above cited articles of
Van Dijk-Wolthuis et al. in Macromolecules 28, and

20   Hennink et al. in J. Contr. Rel. 39). It is also possible
to use polymerization by gamma irradiation, with the
advantage that no initiator and/or catalyst residues have
to be extracted from the hydrogel.

        The hydrogel system of the present invention can

25   easily be tailored with respect to protein drug release
kinetics, which tremendously expand the applicability of
protein drugs. Especially, in the case of biological
response modifiers with a narrow therapeutic window, which
are useful in the treatment of various diseases wherein

30   the immune system is involved, this is very important.

        An increasing degree of substitution (DS; amount of
hydrolysable spacer containing crosslinkable branches per
100 main water-soluble polymer residues; determinable by
^{1}H-NMR) yields a more crosslinked network. This results in

35   a slower swelling rate and an increasing dissolution time
of the gel.

The hydrogels of the present invention can be
prepared in such a way that dissolution times from less
than 1 day upto about 3 months and longer can be obtained.
This can for instance be effected by varying the initial
5   water content in the aqueous polymer solution to be
crosslinked and the DS. Gels with a high initial water
content, such as water contents higher than 85 wt.%
predominantly contain intramolecular crosslinks, while
lower initial water contents give more intermolecular
10   crosslinking. Gels with less intermolecular crosslinks
dissolve faster at the same DS.

Drugs can be loaded into hydrogels either by
equilibration in a drug-containing solution (see e.g.
Kim et al. in Pharm. Res. 9(3) (1992) 283-290) or by
15   incorporation of the drug during the preparation of
hydrogel (see e.g. Heller et al. in Biomaterials 4 (1983)
262-266).

Loading by equilibration, however, normally leads to
a rather low drug content in the delivery system. This is
20   especially the case, when the drug is a macromolecular
compound. Unless the pore size of the hydrogel is rather
large, the macromolecules will only adhere to the outer
surface, which may lead to a burst release.

Therefore, preferably, the drug is loaded during
25   polymerization or crosslinking.

Since microsphere suspensions are easy to prepare and
are easily used for injection, the hydrogels generally
will be applied as microspheres of varying sizes.
Microspheres can be prepared by dissolving
30   HEMA-derivatized dextran in water after which this
solution is added to an oil phase (e.g. silicone oil)
yielding a water-in-oil emulsion. After addition of a
suitable initiator system, the methacrylate groups
polymerize, yielding stable micropheres.
35   The drugs are released from the biodegradable
hydrogels of the present invention during hydrolysis of
the hydrogel, although at least to some extent diffusion

of proteins from the hydrogel takes place. In fact, the
hydrolysis behaviour of the hydrogel and the time during
which compounds present in the hydrogel system are
released can be adjusted to one another so that the
5   release can take place at any level between first order
(no degradation of the hydrogel) and zero order release
(fully degradation controlled) (see in this respect
Example 6, herein-below). This provides evident advantage
over hydrogel systems that are not hydrolysable at
10   physiological conditions, but which are rather stable,
such as the known dextran-GMA hydrogel system, or over
systems wherein the polymers in the hydrogel are elongated
in one dimension only.

        Protein drugs are released from the rather stable
15   hydrogels following first order kinetics - protein release
proportional to the square root of time - which is common
for monolithic delivery systems. The hydrogels of the
present invention, however, show a more zero order release
behaviour - protein release proportional to time. When the
20   hydrogel degrades during the release process, the
diffusion coefficient of the protein drug, present in the
hydrogel, increases. This leads to a more constant release
in time.

        The hydrogel system of the present invention offers
25   - as said - the possibility to tailor the release profiles
of encapsulated protein drugs. More in detail, the
degradation rate of the hydrogel can be adjusted by
varying the water content of the hydrogel, the degree of
substitution, the number and length of hydrolysable groups
30   in the spacers, and the choice of hydrolysable spacers.

        It has been found that spacers based on glycolic acid
have a higher hydrolytical sensitivity than spacers based
on lactic acid. If glycolic acid based spacers are present
an accelerated degradation rate of the hydrogel is
35   observed as compared with lactic acid based spacers.

The effect of the water content of the hydrogel and the degree of substitution of the hydrogel polymers is elaborated in the examples following below.

Further, the rate of release depends on the size of
5 the hydrogel particles. This size can be adjusted by varying the stirring speed, viscosity of the external phase etc. during the preparation of microspheres.

The rate of release does not depend on the length of the water soluble polymers, at least not to a high extent.
10 This is contrary to hydrogel systems wherein the hydrolysable groups are present in the main chains of the polymer only (one dimensionally elongated polymers).

As indicated herein-above, the releasable compound can be a protein drug. However, it is also possible to
15 encapsulate pharmacon containing nanoparticles, e.g. liposomes, iscoms, polylactic acid particles, polycaprolacton particles and gene delivery systems known to the person skilled in the art. The encapsulation of these nanoparticles has the advantage of preventing the
20 occurence of a too fast release of the encapsulated compound, or, said in other words, burst-effects can be avoided in a more secure way.

An example of a loaded hydrogel system within the present invention is a hydrogel containing the cytokine
25 interleukin-2 (IL-2). IL-2 is a protein drug which can e.g. be used in the treatment of particular types of cancer.

For IL-2 to be therapeutically effective in cancer treatment, prolonged presence of IL-2 at the site of tumor
30 growth is required. This can be achieved either by administering high doses of IL-2 intravenously through frequent bolus injections (see e.g. Rosenberg et al. JAMA 271, (1994) 907-913), by prolonged continuous infusion (see e.g. West et al., N. Engl. J. Med. 316, (1987),
35 898-905, or by frequently administering low doses of IL-2 intra- or peri-tumorally (see e.g. Den Otter et al., Anticancer Res. 13, (1993), 2453-2455).

A major disadvantage of the intravenous route is that for obtaining sufficiently high levels of IL-2 at the site of tumor growth, such high doses of IL-2 have to be administered intravenously, that it becomes severely

5 toxic. In contrast, the intra- or peri-tumoral approach, as developed by Den Otter et al., has proven to be very successful and virtually non-toxic in various transplanted and spontaneous tumors.

A serious problem for application of this form of

10 therapy in human cancer patients, however, is that IL-2 has to be injected intra- or peri-tumorally 5 to 10 times within 1 to 2 weeks. For many types of cancer this is not-acceptable burden for the patient, like in cases of lung carcinoma, bladder cancer, and gastric cancer. In first

15 attempts to translate the very effective local, low-dose IL-2 treatment to the human cancer clinic, these logistic problems of IL-2 delivery were already run into. The slow-release delivery system of the present invention makes the use of local IL-2 immunotherapies possible.

20 For the *in vivo* application hydrogel suspensions (microspheres) will normally contain up to 10^5 I.U. of IL-2 in 0.5 ml, which are released over a period of 5 days (i.e. 2×10^4 I.U. of IL-2 released per day). The amount of protein released can be determined with sensitive

25 quantitative detection methods (HPLC, ELISA assays). To investigate whether the released IL-2 is still biologically active and to what extent (i.e. what is the effect of these chemical procedures on the specific activity of IL-2), proliferation assays using the IL-2

30 dependent CTLL cell line can be performed.

The present invention will now be explained in more detail, while referring to the following, non-limiting examples.

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Examples

        To obtain a dextran hydrogel, first a polymerizable
methacrylate group was introduced in dextran. For all
reactions described below, dextran from Fluka (T40,
5   M_n = 15.000, M_w = 32.500 g/mol) was used. Four different
dextran derivatives were synthesized (Examples 1-4) in
which the methacrylate ester was coupled via a spacer to
dextran. The spacer contains different hydrolyzable bonds
(carbonate and/or carboxylic ester). The degradation
10   behaviour of gels prepared from these derivatives was
compared with dextran gels derived from
glycidylmethacrylate (dex-GMA). In this reference
compound, the methacrylate ester is directly coupled to a
hydroxyl group of dextran. This reference gel degrades
15   extremely slowly.

Example 1: Synthesis of dex-HEMA
        Dextran derivatized with hydroxyethyl methacrylate
(dex-HEMA) was synthesized by coupling carbonyldiimidazole
(CDI) activated HEMA (HEMA-CI) to dextran.
20   CDI (1.62 g; 10 mmol) was dissolved in about 10 ml
anhydrous tetrahydrofuran (THF). This solution was added
to a solution of HEMA (1.30 g; 10 mmol) in 5 ml anhydrous
THF. The reaction mixture was stirred for 16 hours at room
temperature. After evaporation of the solvent a slightly
25   yellow liquid was obtained (yield 2.93 g). The crude
product was dissolved in ethylacetate, extracted with
water to remove imidazole and unreacted HEMA and dried on
anhydrous $MgSO_4$. After filtration, the solvent was
evaporated and almost pure hydroxyethyl methacrylate
30   activated with CDI (HEMA-CI) was obtained. The structure
of this product was confirmed by NMR and IR spectroscopy.
        Varying amounts of HEMA-CI (0.73, 1.49, or 2.19 g;
96% pure) were added to a solution of dextran (10 g,
.62 mmole glucose units) and N,N-dimethylaminopyridine
35   (DMAP; 2 g, 16.4 mmol) in anhydrous dimethylsulfoxide

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(DMSO; 90 ml). These reaction mixtures were stirred for
4 days at room temperature after which the reaction was
terminated by the addition of about 2 ml of concentrated
HCl. The reaction mixture was dialyzed against water for
5   3 days at 4°C. Dex-HEMA was isolated by lyophilization
yielding a white fluffy material (yield >90%). The degree
of HEMA substitution was determined by NMR spectroscopy,
and amounted 4, 9, and 13, respectively.

Example 2: Synthesis of dex-SA-HEMA
10   Dextran derivatized with the hemi-ester of succinic
acid (SA) and HEMA (dex-SA-HEMA) was synthesized as
follows.
         SA (2.00 g, 20 mmol), HEMA (2,6 g, 20 mmol),
triethylamine (TEA; 0.28 ml, 2 mmol) and hydrochinon
15   (inhibitor, ±10 mg) were dissolved in about 30 ml
anhydrous THF. The reaction mixture was stirred for 2 days
at 45°C, after which the solvent was evaporated. A yellow
liquid was obtained (yield 4.88 g). The structure of
HEMA-SA was confirmed by NMR and IR spectroscopy.
20   HEMA-SA (0.99 g (94% pure), 4 mmol) and
dicyclohexylcarbodiimide (DCC; 0.83 g, 4 mmol) were
dissolved in about 20 ml anhydrous DMSO. After 15 minutes
a precipitate was formed (dicyclohexylureum; DCU) and this
mixture was added to a solution of dextran (2.57 g,
25   16 mmol glucose units) and TEA (0.56 ml, 4 mmol) in
anhydrous DMSO (20 ml). The resulting mixture was stirred
for 3 days at room temperature, after which 3 drops of
concentrated HCl were added to terminate the reaction.
Next, the reaction mixture was filtered to remove DCU and
30   dialyzed for 3 days at 4°C. After lyophilization, a white
fluffy product was obtained (yield 2.78 g). The degree of
substitution was established by NMR spectroscopy and
amounted to 3.

Example 3: Synthesis of dex-lactate-HEMA

Dextran derivatized with HEMA-oligolactide was
synthesized as follows as illustrated in scheme 1. Three
steps can be distinguished.

5 a. coupling of lactate to HEMA yielding HEMA-lactate;
   b. activation of HEMA-lactate using CDI yielding HEMA-
      lactate-CI
   c. coupling of HEMA-lactate-CI to dextran.

A mixture of L-lactide (4.32 g; 30 mmol) and HEMA
10 (3.90 g; 30 mmol) was heated to 110°C. Thereafter, a
catalytic amount of stannous octoate (SnOct$_2$; 121.5 mg,
0.3 mmol) dissolved in about 0.5 ml toluene was added. The
resulting mixture was stirred for 1 hour. After cooling
down to room temperature, the mixture was dissolved in THF
15 (20 ml). This solution was dropped in water (180 ml) and
the formed precipitate was isolated by centrifugation. The
pellet was taken up in ethyl acetate (40 ml),
centrifugated to remove solid material, dried (MgSO$_4$) and
filtered. The solvent was evaporated yielding a viscous
20 oil (3.74 g, 45%). The product (HEMA-lactate) was
characterized by NMR and IR spectroscopy.

HEMA-lactate (3.74 g, 10.8 mmol) was added to a
solution of CDI (1.76 g, 10.8 mmol) in THF and stirred for
16 hours at room temperature. The solvent was evaporated
25 under reduced pressure yielding a viscous oil. The product
containing HEMA-lactate-CI and imidazole as major
compounds (NMR analysis) was used without further
purification.

To a solution of dextran (10 g, 62 mmol glucose
30 units) and DMAP (2.0 g, 10.6 mmol) a varying amount of
HEMA-lactate-CI was added (1.61, 3.23 or 4.84 g
respectively, 80% pure). These mixtures were stirred for
4 days at room temperature after which the reaction was
terminated by the addition of about 2 ml of concentrated
35 HCl. The solutions were dialyzed against water for 2 days.

After lyophilization, white fluffy products were obtained
(yield around 85%). The degree of substitution (as
determined by NMR spectroscopy) amounted to 3, 6 and 10
for the three products, respectively.

5   Using similar procedures, the length of the lactate
spacer can be varied by changing the molar ratio of HEMA
and lactide in the first reaction.

Example 4: Synthesis of dex-glycolide-HEMA
           Dex-glycolide-HEMA was synthesized according to the
10   same procedure as described in Example 3 replacing lactide
by glycolide.

Reference Example 1: Dex-GMA
           Dex-GMA was synthesized as described by Van Dijk-
Wolthuis et al., Macromolecules 28, (1995), 6317-6322. An
15   reinvestigation of the obtained dextran derivative
revealed that the methacrylic ester group is directly
coupled to one of the hydroxylic group of dextran, meaning
that the glyceryl spacer is not present.

Example 5: Preparation of dextran hydrogels
20   Hydrogels were obtained by a free radical
polymerization of aqueous solutions of methacrylated
dextran (prepared according to Examples 1-4 and
Ref. Example 1). Methacrylated dextran (100 mg) was
dissolved in 760 µl PBS buffer (10 mM phosphate, 0.9%
25   NaCl, 0.02% NaN$_3$, pH 7.2). To this solution 90 µl of
potassium peroxydisulfate (KPS; 50 mg/ml) in the same
buffer was added per gram solution and mixed well.
Subsequently, N,N,N'N'-tetramethylethylenediamine (TEMED;
50 µl; 20% (v/v) in water, pH adjusted to 7) was added and
30   the resulting solution was quickly transferred into an
Eppendorf tube and polymerized for 1 hour at room
temperature yielding a hydrogel material with an initial
water content of about 90% (w/w) after polymerization.

The gels were removed from the tubes, accurately
weighed and incubated in PBS at 37°C. Periodically, the
weight of the gels was determined and used to calculate
the degree of swelling (= W_t/W_o, in which W_t is the weight
5   of the gel at time t and W_o is the initial weight of the
gel). The hydrogel degradation (dissolution) time is
defined as the time at which the swelling ratio = 0
(or W_t = 0).

Figure 1 shows the swelling behaviour of three
10   different dextran hydrogels (initial water content 90%).
The dex-GMA (DS = 4) reached an equilibrium swelling
within 3 days; thereafter the weight of the gels remained
constant indicating that no significant hydrolysis of
methacrylate esters occurred. Dex-HEMA and dex-lactate-
15   HEMA showed a progressive swelling in time until these
gels dissolved completely. This demonstrates that in these
hydrogel systems hydrolysis of carbonate esters (dex-
(lactate)HEMA) and/or lactate esters (dex-lactateHEMA)
occurred.

20   Figure 2 shows the swelling behaviour of dex-
lactateHEMA hydrogels (initial water content 92%) and
varying degree of substitution. As can be seen an
increasing DS resulted in an increasing dissolution time.

Figure 3 shows the swelling behaviour of dex-
25   lactateHEMA hydrogels with a fixed DS (6) and varying
initial water content. It appears that the dissolution
time increases with an increasing initial water content.

Figure 4 shows the swelling behaviour of dex-SAHEMA
hydrogel (DS 3) and a varying initial water content.

The next tables give an overview of the dissolution times of different dextran hydrogels

dissolution time (days) of dex-lactate-HEMA gels

initial water content	DS 10	DS 6	DS 2.5
90%	8-13	4	1-2
80%	16	7-18	3-9
70%	22	10-19	6-10

dissolution time (days) of dex-HEMA gels

initial water content	DS 8	DS 17	DS 20
92%	25	42	55
80%	53	100	ND
67%	70	>100	ND

dissolution time (days) of dex-HEMA-SA gels

initial water content	DS 3
80%	15
74%	23-28
60%	44

The dissolution time of dextran hydrogels (initial water content 92%) obtained by polymerization of dextran derivatized with GMA (DS 4) had a dissolution time of about 100 days (pH 7.2, 37°C). Gels obtained by polymerization of dextran derivatized with GMA (initial water content 80%, DS 11) did not show any signs of degradation (increased swelling) during 70 days, even at extreme conditions (37°C, pH 12 and pH 1).

Example 6: Protein release from degrading dextran hydrogels

The release from non-degrading dex-GMA hydrogels has been studied extensively. It appeared that when the protein diameter was smaller than the hydrogel mesh size, the release of the protein could be effectively described by the free volume theory. In this case the cumulative amount of protein released was proportional to the square

root of time (W.E. Hennink, Controlled release of proteins
from dextran hydrogels, Journal of Controlled Release 39,
(1996), 47-55).

5   Figure 5 shows the release of a model protein (IgG)
from degrading dextran hydrogels (dex-lactate-HEMA, DS
2.5). The release of IgG from these degrading gels is zero
order (cumulative release proportional to time). This is
in contrast with the release of proteins from non-
degrading dextran hydrogels, where a first order release
10   has been observed.